Defensin

Week 1

Summarized below are the experiments conducted this week in chronological order. Click on the experiment name to view it. To go back to this summary, click **Summary** in the footer.

Summary

1 Transformation of pSB1C3-Def with Colony PCR analysis sequencing analysis, and inoculation in liquid media

Responsible

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Protocols used

PCR Amplification
PCR Purification

Modifications and comments to protocols

Q5 PCR Amplification primers:	VF2_R (reverse)
	VF2_F (forward)
PCR Purification	50 μl Buffer EB (first attempt)
	30 μl Buffer EB (second attempt)

Experimental Set Up

Table 1: Volumes of components in PCR master mix for 18 samples (not only for this experiment).

Component	Volume μl]
10 μM VF2_F	18
10 μM VF2_R	18
10x PCR Buffer	90
Taq DNA Polymerase	4.5
dNTP mix	18
ddH_2O	751.5

Table 2: Volumes for glycerol stock of OD_{600} 0.6 cell culture.

Component	Volume μl]
Cell culture	250
Glycerol (50 %)	250

Results and Conclusions

The transformation was successful and four Def colonies were picked, Def1-4.

Table 3: Concentrations of PCR purified samples (first attempt).

Sample	Concentration [ng/μl]
Def1	8.4
Def2	12.9

The concentration of the samples are too low for sequencing.

Table 4: Concentrations of PCR purified samples (second attempt).

Sample	Concentration [ng/μl]
Def1	17.6
Def2	20.3

The concentrations are high enough use for sequencing analysis.

Discussion and Troubleshooting

Elution of 50 μl DNA sample with 50 μl Buffer EB were not the optimum for maximizing the final concentration in the elute. By eluting with a smaller volume of Buffer EB, a higher final concentration was achieved.